Editorial

Genotyping, Clopidogrel Metabolism, and the Search for the Therapeutic Window of Thienopyridines

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It is somewhat remarkable to note that not until recently, nearly 12 years and tens of millions of treated patients after its initial approval, has the metabolism of clopidogrel, an inactive prodrug, begun to be more fully understood. Although this lack of knowledge may not be unique among common cardiovascular drugs, there are 4 characteristics of clopidogrel therapy that make this gap in understanding disconcerting: The potential for life-threatening complications (bleeding or thrombosis) at both ends of its therapeutic spectrum; the fact that 1 dose is supposed to fit all; the lack of a measureable, proven end point (such as blood pressure for antihypertensive drugs) that allows for titration to a target response; and the nearly mandatory need by many patients for clopidogrel, with no acceptable alternative until recently.

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Historically, understanding the metabolism of a drug has been critical in identifying those patients most likely to benefit from and, more important, potentially be harmed by that drug. This is especially true for drugs with relatively narrow therapeutic windows, such as phenytoin, warfarin, and others. Although it seems safe to assume, on the basis of our extensive clinical experience, that the therapeutic window of clopidogrel is unlikely to be narrow, this certainly does not represent the depth of knowledge we would hope for after so much clinical experience and research. What we do know is that P2Y12 inhibition with thienopyridines is beneficial in preventing arterial thrombotic events, presumably requiring the achievement of some minimal level of receptor inhibition that may or may not vary on the basis of the patient and clinical scenario. We also know that it is associated with a significant increase in serious bleeding events, with higher levels of inhibition associated with greater bleeding. In the present and future practice environment, with a growing presence of P2Y12 inhibitor options and the requirement to better individualize therapies, improved methods for identifying outlier patients who are at increased risk of harm with standard dosing of clopidogrel are critically needed.

Given that only an estimated 2% of ingested clopidogrel ends up bound to platelets,[1] it is easy to appreciate that small changes in its metabolism may substantially affect platelet P2Y12 inhibition. Hepatic metabolism is essential to the generation of the active metabolite of clopidogrel, specifically, 2 sequential oxidative steps through the cytochrome P450 (CYP) system.[2] This superfamily of proteins, which encompasses 57 genes and 18 families, is involved in ≈80% of oxidative drug metabolism, as well as the metabolism of many other exogenous and endogenous chemicals, including arachidonic acid and eicosanoids, steroids, lipids, bile acids, and vitamins.[3] We now know that a variety of P450 enzymes contribute to clopidogrel metabolism. The first metabolic step, which leads to 2-oxo-clopidogrel, is catalyzed by 3 enzymes (CYP1A2, CYP2B6, and CYP2C19), whereas the second step, which culminates in the active metabolite, involves 4 enzymes (CYP2B6, CYP2C9, CYP2C19, and CYP3A4).[2] With the CYP2C19 enzyme involved in both steps, contributing to an estimated 45% of 2-oxo-clopidogrel generated and 21% of its conversion to active metabolite, it is understandable why so much recent research has focused on the potential impact of drugs and genetic polymorphisms that influence the activity of this enzyme.

To date, 25 polymorphic variants of the *CYP2C19* gene have been identified, with the frequency of variant alleles varying among different ethnic groups.[4] One of the most common, with an allelic frequency of ≈15% to 30%, is designated CYP2C19*2. This polymorphism causes a splicing defect and a complete loss in enzyme activity, presenting phenotypically as a poor metabolizer. A number of recent mechanistic and clinical studies have found a relatively consistent association with the presence of this allele and a decrease in ex vivo–determined platelet function, which appears to be related solely to clopidogrel response,[5] as well as worse clinical outcomes in clopidogrel-treated patients.[6–8] In an analysis in the present issue of *Circulation*, Sibbing and colleagues[9] are the first to specifically explore the other side of CYP2C19 activity, phenotypic ultrarapid metabolizers due to the presence of the CYP2C19*17 polymorphism, which also has a relatively high allelic frequency of ≈4% to 18%. They did this by analyzing platelet function and clinical outcomes in more than 1500 clopidogrel-treated percutaneous coronary intervention patients. The mechanistic expectation would be that the presence of this polymorphism would contribute to an increase in the generation of the active metabolite of clopidogrel, and with it, higher levels of platelet inhibition, and therefore potentially greater efficacy but also more bleeding. The investigators found that the presence of the *17 polymorphism was indeed associated with a higher level of platelet inhibition, as measured with multiple-electrode platelet aggregometry, in a gene-dose–dependent manner (ie, homozygotes had greater platelet inhibition than heterozygotes, who had greater inhibition than wild types).

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

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Although they also confirmed a *17 dose–dependent increase in the incidence of Thrombolysis in Myocardial Infarction (TIMI) major and minor bleeding, no association was found for improved efficacy. Although the number of stent thrombosis events (14) would be too small to reach much of a conclusion regarding a lack of increased efficacy in *17 carriers, the number of combined ischemic events (death/myocardial infarction/urgent target-vessel revascularization) at 30 days (56) was even greater than the number of TIMI bleeding events (51).

Taken at face value, the totality of all of the CYP2C19 polymorphism data may suggest that it would be appropriate to begin genotyping all potential thienopyridine-eligible patients and thus identify those patients who would be at increased risk for thrombosis or bleeding if treated with clopidogrel. But is it that simple? Does knowing the genetic makeup of 1 CYP enzyme provide enough information to identify patients who will fall outside the therapeutic window of clopidogrel and justify altering therapy?

Because all biological processes reflect the combined influence of multiple environmental and genetic inputs, it is important to consider the metabolism of clopidogrel and in particular the role of 2C19 as a single piece of a complicated puzzle. Consistent with this, in 1 analysis, the CYP2C19*2 genotype was able to explain only 12% of the variation in response to clopidogrel, and this was in a uniquely homogeneous and healthy white population taking no other medications.[5] This modest, although not unimportant, effect likely reflects the great diversity of possible environmental and genetic factors affecting the numerous steps involved in the generation of the active metabolite of clopidogrel (Table).

Every patient represents a unique and nonstatic combination of many influences (genetics, concomitant disease processes, medications, foods, age, and lifestyle), all of which culminate in variations not only in clopidogrel active-metabolite formation but also in all aspects of measured on-treatment platelet function. For example, 17 novel single-nucleotide polymorphisms appear to account for just half of the large variability in platelet function measured even off-treatment,[10] which strongly correlates with on-treatment platelet function.[11] Similarly, a number of clinical factors (increasing age, acute coronary syndrome, decreased left ventricular function, diabetes mellitus, and renal insufficiency) are also independently associated with on-treatment platelet function.[12] The innumerable combinations of genetic and environmental inputs is at least a partial explanation as to why a majority of CYP2C19*2 carriers are still found to be "clopidogrel responsive,"[13] why the randomized trial data do not support a clinically important impact of acquired CYP losses of function with lipophilic statins[14] and proton pump inhibitors,[15] and, pertinent to the present analysis, why previous studies of CYP2C19*17 have been unable to identify an association with either platelet aggregation or bleeding.[5,8]

With such tremendously diverse and numerous influences affecting platelet function during treatment with a thienopyridine and aspirin, there remains a critical need to identify a phenotypic test of platelet-dependent thrombosis that not only identifies risk but also much more important is proven to be able to isolate a specific contributor to that risk—the degree

Table. Genetic and Environmental Factors That Can Each Potentially Influence the Generation of the Active Metabolite of Clopidogrel

Absorption (estimated to be at least 50% of administered dose)
Patient compliance
Proximity to and type of meal
Previous gastric surgery
Transmembrane transporter MDR1/P-glycoprotein (also called ABCB1)
Genetic polymorphisms: >40 identified SNPs
Drug inducers (eg, colchicine, insulin, methotrexate)
Environmental and drug inhibitors (eg, orange and grapefruit juices, green tea, garlic, quinidine, nicardipine, amiodarone)
Crushed vs whole tablet
Metabolism (because metabolism is primarily hepatic, all processes below are influenced by liver disease; they are also differentially influenced by underlying inflammatory states)
Hydrolysis (converts ≈85% of absorbed parent drug to clopidogrel carboxylate, an inactive metabolite)
HCE 1
Genetic polymorphisms: 16 indentified SNPs
Drug inhibitors (eg, procainamide)
Alcohol ingestion
First oxidative step (conversion of clopidogrel to 2-oxo-clopidogrel)
CYP1A2 (responsible for ≈36% of conversion)
Genetic polymorphisms: 16 identified SNPs
Environmental and drug inducers (eg, tobacco, char-grilled meat, insulin, omeprazole, St John's wort)
Environmental and drug inhibitors (eg, caffeine, grapefruit juice, verapamil, fluoroquinolones)
CYP2B6 (responsible for ≈19% of conversion)
Genetic polymorphisms: 29 identified SNPs
Drug inducers (eg, carbamazepine, glucocorticoids, rifampin)
Drug inhibitors (eg, SSRIs, estradiol)
CYP2C19 (responsible for ≈45% of conversion)
Genetic polymorphisms: 25 identified SNPs
Drug inducers, eg, prednisone, rifampin, St John's wort
Drug inhibitors, eg, proton pump inhibitors, SSRIs, cimetidine, indomethacin
Second oxidative step (conversion of 2-oxo-clopidogrel to the active metabolite)
CYP2B6 (responsible for ≈33% of conversion)
Genetic and drug influences: see above
CYP2C9 (responsible for ≈7% of conversion)
Genetic polymorphisms: 34 identified SNPs
Drug inducers (eg, carbamazepine, rifampin, St John's wort)
Drug inhibitors (eg, fluconazole, valproic acid, amiodarone)
CYP2C19 (responsible for ≈20% of conversion)
Genetic and drug influences: see above
CYP3A4 (responsible for ≈40% of conversion)
Genetic polymorphisms: 20 identified SNPs
Drug inducers (eg, aspirin, barbiturates, rifampin, St John's wort)
Environmental and drug inhibitors (eg, verapamil, lipophilic statins, macrolide antibiotics, fluconazole, protease inhibitors, grapefruit juice, star fruit)

SNP indicates single-nucleotide polymorphism; HCE, human carboxylesterase; and SSRI, selective serotonin reuptake inhibitor.

SNP data are taken from http://www.cypalleles.ki.se.

Percent conversion data are from Kazui et al.[2]

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of P2Y12 inhibition—and by doing so allow therapy to be titrated to maintain a patient within a yet unidentified therapeutic window of P2Y12 inhibition that maximizes efficacy while maintaining safety. There are several ongoing trials (NCT00645918, NCT00774475, and NCT00827411 in the ClinicalTrials.gov registry) evaluating whether present techniques are able to accomplish this, and their results will be a great leap forward.

Although genotyping may play a partial clinical role as 1 of several factors to predict platelet function during clopidogrel treatment until an adequate phenotypic test is identified, its more prominent role will likely remain as an extremely valuable research tool that provides mechanistic guidance and hypothesis-generating clues to diseases and their treatment. For example, it is interesting to note that in the present analysis by Sibbing et al,[9] higher levels of active-metabolite generation led to increased bleeding but no increase in efficacy, which somewhat mirrors the CYP2C19*2 analyses from TRITON (Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel) when clopidogrel- and prasugrel-randomized patients are compared side by side.[7,16] Although highly speculative, both genotypic analyses support the possibility that, like warfarin, beyond a certain cutoff, increasing levels of inhibition may not improve efficacy but only increase bleeding, which suggests that the therapeutic window for thienopyridines may not be that broad after all.

Disclosures

Dr Steinhubl is a full-time employee of The Medicines Company.

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